FURTHER RESEARCHES ON PARASITIC PROTOZOA FOUND IN CANCEROUS TUMOURS.

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(Plates I. to IV.)

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Section II.

We have seen in the preceding section that the protozoa infecting the cancerous cells of the breast[1] occur in the nucleus, but are to be found mainly in the protoplasm; hence the necessity for cutting a large number of sections in order to find the parasite inside the nucleus. The comparative rarity of the protozoon in this situation may account for the fact that Sawtschenko, Foà, and others, have not observed it in the nucleus, though it is probable that some of the bodies found by Thoma, and especially by Steinhaus, in the nucleus of the cancer cell were really parasites.

The structure of the parasite, as it is observed in the infected epithelial cell of breast cancers, must now be discussed at length. The central, more darkly-staining part of the parasite we have shortly called the nucleus, because, when seen in hardened specimens, it resembles in structure the nuclei of other protozoa; but it is probable that this represents but a small part of the nucleus. It differs, however, from the nuclei of the epithelial cells in its micro-chemical reactions, as also in its structure.

In the first place, the nucleus of the parasite—for brevity's sake we shall refer to it in future as "the nucleus"—does not stain easily with the ordinary nuclear dyes. This aversion to nuclear dyes is found in other protozoa also, after these have been submitted to the action of hardening reagents, and some light is thrown on this question by the

[1] Since the first section of the paper was written, we have completed the examination of fifty cases of cancer of the breast, in all of which we have found the above-described protozoa. We do not include in these fifty cases several metastases of breast cancers in the glands and internal organs, nor a case of cancer of the male breast, all of which were similarly affected.

reaction observed in the coccidia infesting the biliary ducts of the rabbit, the nuclei of these parasites also having no affinity for nuclear dyes.

R. Pfeiffer[1] has made a similar observation, for, in his paper on the coccidia of the rabbit, he states: "Der Kern ist schwer zu sehen, und erscheint, wenn er erkennbar ist, wie eine hellere rundliche Vacuole ohne jede Struktur. Bei Färbungen mit den gewöhnlichen Kernfärbungsmitteln färbt sich das Protoplasma dieser Körperchen schwach. *Der nucleus*[2] *bleibt ganz ungefärbt und stellt sich daher als relativ grosse, runde Lücke dar, in deren inneren ein stark gefärbter sehr grosser, völlig runder Nucleolus hervortritt.*"

Similarly, it is extremely difficult to stain the nucleus of the cancer parasite with hæmatoxylin, and Burchardt,[3] working under Recklinghausen, is of opinion that the parasites described by him in one case of colloid cancer are "absolut refraktär" towards hæmatoxylin. Personally we would not go quite so far. It is quite true that hæmatoxylin stains such parasites only with great difficulty, nevertheless it is possible to do so in specimens fixed in osmic acid solution, or in Flemming's fixing fluid, especially if Delafield's staining solution be employed. But even in successful cases the nucleus as a rule takes the same colour as the protoplasm of the cell, and is always sharply differentiated from the nucleus of the epithelial cell. In other cases it shows the peculiar phenomena of metachromatosis, described by Soudakewitch and by Ruffer and Walker, the nucleus of the epithelial cell remaining blue and the nucleus of the parasite violet-coloured.

Ruffer and Walker[4] had already drawn attention to the fact that the protoplasmic substance of the large parasites in a case of cancer of the stomach often stained Cambridge-blue with the Ehrlich-Biondi mixture, whilst the nucleus stained red, the nucleolus of the epithelial cell also assuming the same colour. In the breast such marked metachromatism of the protoplasm of the parasite is but rarely observed. The nucleus, however, nearly always stains more or less brilliantly red, as it takes up the acid fuchsine contained in the solution.

A similar reaction may sometimes be obtained with Löffler's blue in osmic acid preparations. The nucleus of the epithelial cell is of a faint, dirty-green colour, the nucleus of the parasite dark blue. If, on the other hand, sections be made from a cancer hardened in chromic acid and spirit, and stained for twelve hours in eosin and then in aniline blue for a few minutes, washed in water, dehydrated in alcohol, and mounted in balsam, *secundum artem*, the parasite remains intensely blue, even when all the aniline blue has been washed out, both from

[1] R. Pfeiffer, "Beiträge zur Protozoen-Forschung, Heft 1. Die Coccidien Krankheit der Kaninchen," 1892, p. 5.

[2] The italics are ours.

[3] Burchardt, see preceding section, p. 125.

[4] M. Armand Ruffer and J. Herbert Walker, *Journal of Pathology and Bacteriology*, vol. i. p. 205.

the protoplasm and from the nucleus of the cancer cell (see Plate II., Fig. 35).

It is possible to obtain a similar differentiation in sections fixed in chromicised spirit, by first staining by Gram's method. After the usual iodine solution has been allowed to act, the section is stained for a sufficient length of time in a 1 per cent. solution of acid fuchsine. It is then passed through alcohol, aniline-xylol, xylol, and mounted in Canada balsam. In sections carefully prepared in this manner, the nuclei of the epithelial cells are purple, the protoplasm rosy-red, the parasite and the fibrous tissue red (see Plate I., Fig. 2).

Another method which we have used successfully in order to obtain a differentiation is as follows:—Fix in chromicised spirit, or Flemming's solution, and harden in alcohol of gradually increasing strength. Soak the sections in a saturated solution of iron-alum for twelve hours, at a temperature of 38° C., wash well in water, stain in 1 per cent. aqueous solution of hæmatoxylin until the section turns black, place in hydrochloric acid solution, 1 to 500, until the section turns yellow, and then in saturated solution of carbonate of lithium until it becomes blue. Now stain with a saturated solution of cochineal in water, wash out with alcohol, clear in clove oil or xylol, and mount in xylol-balsam. In sections so treated, the parasite and the fibrous tissue stain a brown-red with the cochineal, whilst the nucleus of the epithelial cell and the nucleolus stain blue with the hæmatoxylin. Should cochineal not be used, and the section be deeply stained in aqueous hæmatoxylin alone, the nucleus of the parasite retains the hæmatoxylin and shows well-marked metachromatosis. It seizes upon the cochineal at once if this be allowed to act afterwards, and the colour of the parasite will depend on the concentration of the cochineal stain.

As one of us[1] has already pointed out, the nucleus of the parasite retains the acid fuchsine in specimens fixed in Flemming's fluid and stained with methyl green and acid fuchsine.

The nucleus of the parasite is therefore in many respects different in its micro-chemical reactions from the nucleus of the cancer cell; a distinction not without importance, as we shall perceive presently. To some extent it reacts to aniline dyes, like the nucleolus of the epithelial cell, as Ruffer and Walker have already pointed out, but the reactions, nevertheless, differ in several particulars. Thus, with hæmatoxylin and cochineal, the nucleolus of the epithelial cell stains blue, whilst the parasitic nucleus takes up the cochineal. A more striking example of this is seen in specimens fixed in picric acid, or chromicised spirit, and stained with eosin, and afterwards with aniline blue. If the section be now washed in water, and the process be watched under the microscope, the first structure which, as the blue dissolves out, appears through the confused mass of staining matter, and which is stained intensely with eosin, is the nucleolus of the epithelial cell; whereas the nucleus of the

[1] Ruffer and Walker, *loc. cit.*

parasite retains the blue, when this has been dissolved out from every other structure except the fibrous tissue.

The nucleus is, in hardened specimens, perfectly homogeneous as a rule, but with very high powers a lighter spot (vacuole?) can most frequently be observed in it, especially when hæmatoxylin and cochineal are used as staining reagents. We have never been able to detect anything like a membrane or any karyokinetic figures when fission occurs in the parasite.

We would here draw attention to the fact that these parasites can be plainly made out in cover-glass preparations, fixed with the vapour of hydrocyanic acid or sublimate solution, and stained with Ehrlich-Biondi's reagent. In such preparations the nuclei stain red.

The nucleus in the *living protozoon* is larger than in the same organisms subjected to the action of fixing fluids. In fresh scrapings, examined in ·75 per cent. salt solution, the parasite can be made out surrounded by its capsule and lying in the interior of the cell; the nucleus appears as a round clear vacuole, undergoes spontaneous movements, and changes its shape during the course of observation. Unfortunately, our work with fresh specimens has for various reasons been delayed, and is not advanced enough for publication.

There is but little to add concerning the protoplasm of the parasite. The radiated appearance, previously described, can be almost always observed as soon as the parasite attains a certain size. When the parasite, for any reason or other, has shrunk within its capsule, these rays start from the periphery of the protoplasm and not from the capsule itself.

The capsule of the parasite requires a few more words of comment, if it were only to set at rest a question left unanswered by Ruffer and Walker in their first paper on the subject. When discussing the mode of formation of the capsule, these observers wrote as follows:[1]— "We are inclined to believe that this capsule is secreted by the invaded cell, and not by the enclosed parasites, as it is continuous with the protoplasm of the cell, and is often quite distinct from the parasite, which sometimes, as we have seen, is perfectly free only in the interior of the cyst." They felt inclined to regard the capsule as a kind of protecting membrane, thrown out by the cell to defend itself against the invading protozoon. The question can only be settled by examining the protozoon freed from any surrounding cellular structures, so it is at present still impossible to say whether this opinion be correct or not, but, when formed, the capsule is certainly part and parcel of the protozoon. This is easily demonstrated when, for some reason or other, the capsule shrinks in the interior of the epithelial cell. (See Plate II., Fig. 19 *a*, Fig. 22 *a*; Plate I., Fig. 7 *a*, etc.) In Plate II., Fig. 19 *a*, for instance, the parasite, capsule and all, lies in a clear space in the epithelial cell, whereas the pseudo-capsule which sur-

[1] Ruffer and Walker, *loc. cit.* p. 206.

rounds the pseudo-parasite of Wickham, is always closely applied to the surrounding cell. (Plate III., Fig. 50 *a*, Fig. 46 *a*, Fig. 52 *a*, Fig. 59 *a*, etc.) In other cases the capsule may be wrinkled and folded over, thus clearly showing its independence. Lastly, and this may be mentioned here, although the observation was made on an abdominal cancer, we have observed the parasite lying free in a cancer-alveolus, still surrounded by its capsule, but without any trace of a cell around it.

The protoplasm of the parasite is either quite homogeneous, or contains a few granules and the rays above mentioned. Sometimes, however, these granules arrange themselves in a beautifully regular manner, close to the capsule of the cell (see Plate I., Fig. 17; Plate II., Figs. 23, 24), or at any rate at the periphery of the protoplasm of the parasite (Fig. 23). On first examination one may feel inclined to assume that the granules so formed are stages of reproduction of the parasite, so beautifully symmetrical is at first their arrangement, and so equal their size. Such a conclusion is not warranted, for in a further stage the nucleus of the parasite undergoes a similar kind of breaking up (Plate I., Fig. 9; Plate II., Fig. 23), whilst the rays above mentioned often become much plainer (Plate I., Fig. 17), and other granules appear scattered in an extremely regular fashion throughout the protoplasm of the parasite (Plate II., Fig. 24, and Plate I., Fig. 11). Later on, the whole protoplasm becomes converted into a mass of granules (Plate I., Fig. 9). On using a very high power, however (Zeiss Apochr. 1·5 mm. comp. Oc. 18), these granules are found to be by no means always regular in shape, or in arrangement (Plate III., Fig. 36 *a*). Moreover, they never seem to develop into anything more, never burst the capsule, never surround themselves with protoplasm or set themselves free, so that although unable to give an altogether satisfactory explanation of the appearances, we cannot allow at present that they have anything to do with the reproduction of the parasite; especially as the possibility of their being artificial products, due to the action of the coagulating reagent, cannot be excluded. We are unable also to account satisfactorily for appearances such as are shown in Plate I., Fig. 17, and Plate II., Fig. 33. These granules are much coarser, much more irregular, and more deeply stained than the nuclei formed during the stages of reproduction of the protozoon.

The parasites vary greatly in size, and some attain very large dimensions, but their size varies in one section from 0·004 mm. to 0·04 mm., or even larger. When they attain this latter magnitude, their capsule becomes much thicker, wrinkled, or variously distorted, and they are rarely perfectly spherical on section, but rather oval or even slightly irregular in shape (Plate II., Fig. 34, and Plate I., Fig. 10), resembling then to a remarkable extent the coccidia found in the rabbit's liver and sheep's intestine.[1] The resemblance becomes even more

[1] Ed. Noeard, "Coccidial Tumours from the Small Intestine of the Sheep," *Journal of Pathology and Bacteriology*, vol. i. p. 404.

striking when the protozoon, having attained its maximum size, no longer fills the capsule, but lies in the middle, or on one side, floating probably in some albuminous fluid, and staining slightly with the ordinary protoplasmic dyes (see Plate I., Figs. 7, 10; Plate II., Fig. 34; Plate III., Fig. 36, etc.). In such cases it not unfrequently breaks up into large round homogeneous clumps of varying size (see Plate I., Fig. 10 *a*), the physiological meaning of which is still not clear.

Section III.

We now pass to what is perhaps one of the most important parts of our subject, namely, the phenomena of division of these parasites, as they have been observed by us in cancer of the breast.

At the discussion which took place before the Pathological Society in London, we briefly indicated the mode of reproduction of the parasites of cancer, and a very short abstract of our researches appeared in the *Compt. rend. de l'Acad. des Sciences* for April 1893,[1] and in the *Compt. rend. de la Soc. de Biologie* for the same month. We may now give our observations in detail, prefacing our remarks by saying that although all the illustrations and descriptions are derived from carcinomata of the mamma, yet similar stages have been observed by us in cancer of the intestinal tract, as well as in epitheliomata of the skin.

In the first place, let us see what has been written on this question by observers who have been working at the same subject:—Wickham[2] has described in some of these cysts which he regards as parasitic, a number of small bodies, flattened against each other. We shall return to this description in the last part of our paper, and simply say here that Borrel[3] has already, in our opinion, shown the fallacy of Wickham's opinion. Nils Sjöbring[4] states that in the parasites which he has described, segmentation takes place into a great many spores, whilst, at the same time, a capsule is developed around the parasites, so that a sporocyst is formed. The spores are at first of the shape of small curved rods with swollen ends, and possess a membrane; later on, they leave this membrane, and become converted into small round bodies, which then invade another cell. We cannot say that our observations bear out Sjöbring's description, although possibly the first stage described in his paper may agree with some of the appearances observed by us.

[1] M. Armand Ruffer et H. G. Plimmer, "Sur le Mode de reproduction des parasites du cancer," *Compt. rend. de la Soc. de Biologie*, 15 Avril 1893, and *Compt. rend. de l'Acad. des Sciences*, 17 Avril 1893.

[2] Wickham, "Anatomie et Nature de la Maladie de Paget du Mamelon," *Arch. de méd. exp. et d'anat. path.* 1890, vol. ii. p. 49.

[3] A. Borrel, "Sur la signification des figures décrites comme coccidies dans les epithéliomes," *ibid.* 1890, p. 786.

[4] Nils Sjöbring, "Ein parasitärer Protozoon-artiger organismus in carcinomen," *Fortschr. d. Medicin*, 1890, No. 16.

Soudakewitch,[1] in his second paper, speaks as follows of falciform bodies observed by him:—"Some of these inclusions presented a mass, for the most part spherical, of colourless protoplasm, on the surface of which two, three, or four intensely staining falciform bodies were arranged. As a rule, the falciform bodies were two in number, and their concave borders were turned against each other at an acute angle, or more frequently they were arranged parallel to each other. The space between the corpuscles contained a structureless and colourless substance. These inclusions were far smaller than a blood corpuscle. The smallest inclusions of this category had the appearance of small protoplasmic masses with a chromatophile granule.

"These forms of inclusions just described were nearly always contained in cells, but in the same tumours I found other forms which, although not contained in cells, had some characteristics in common with the inclusions described. These forms were considerably larger, and their colourless protoplasm was somewhat granular. Chromatophile granules of various forms and shapes were disseminated sometimes on its surface and sometimes through the interior. Some of them showed the falciform appearance already described. The study of the various forms of the inclusions of this kind (falciform bodies) enabled us to establish a certain relationship. These inclusions had but few features in common with the degenerative products of cell protoplasm or cell nuclei. The existence of a well-marked capsule around some of these enclosed bodies seems to be an argument in favour of their parasitic nature."

Let us see what other observers have to say on some of these "falciform bodies," and other like structures occurring in cancer. Podwyssoszki and Sawtschenko[2] have described the protozoa filled with "characteristic falciform embryos." Foà[3] states that in the course of the development of the parasite, the central corpuscle constantly increases and becomes a large lobulated mass, from which a number of homogeneous, highly refractive, smaller corpuscles, which he believes to be spores, become detached. Foà's paper is but a preliminary note, so that we may be allowed to postpone criticism until his full paper is published. We may, however, remark that our observations apparently agree in many particulars, but differ in others. Sawtschenko[4] has described in his second paper clusters of small parasites, which arise

[1] Soudakewitch, "Parasitisme intra-cellulaire des néoplasies cancéreuses," *Annales de l'Institut Pasteur*, 1892, tome vi. p. 553. See also Strœbe's "Referat" in *Centralbl. f. Path. Anat. u. allg. Path.* 1891, Bd. ii. p. 460.

[2] Podwyssoszki and Sawtschenko, "Parasitismus bei Carcinomen nebst Beschreibung einiger schmarotzenden Sporozoen," *Centralbl. f. Bakteriologie u. Parasitenkunde*, 1892, Bd. xi. p. 560.

[3] Pio Foà, "Sul parassiti del cancro," *Estratto della Gazetta Medica di Torino*, 1893, Anno xliv. No. 3, and *Brit. Med. Journ.* "Epitome of Current Literature," 25th Feb. 1893, p. 32.

[4] Sawtschenko, "Weitere Untersuchungen ueber schmarotzenden Sporozoen in den Krebsgeschwülsten," *Centralbl. f. Bakteriologie u. Parasitenkunde*, 1892, Bd. xii. p 17.

through a process of segmentation, starting from the periphery of a larger parasite which then loses its capsule. In other cells,—the so-called physaliphora,—Sawtschenko states that he has found falciform bodies representing, as he thinks, the falciform spores of sporozoa. Metchnikoff,[1] on the other hand, writes as follows concerning these falciform bodies:—"MM. Strœbe, Podwyssoszki, and Sawtschenko, and quite recently M. Soudakewitch, have discovered falciform bodies in several cases of cancer. From all that I have been able to observe up to the present, as well as from all the statements of the authors whom I have just quoted, the formations taken by them for falciform bodies, or (what is the same thing) for stages of the crescent, can in no way be compared with the corresponding productions of coccidia or of sporozoaria in general. . . . I look upon the latter (falciform bodies, etc.) as chromatic degenerations of the nucleus of cancer cells. They may be designated as pseudo-crescents, just as in cancers (especially in epitheliomata) it is necessary to distinguish pseudo-coccidia, so often confounded with formations really analogous to sporozoaria."

L. Pfeiffer,[2] describes the zoospores of cancer as follows:—"This form of zoospore is present in two sizes; the larger is more polymorphous, the smaller is rounded or angular. Both forms have the characteristics of epithelial cells of the type of gland cells, and have a granular protoplasm; their nucleus is large, coarsely granulated with distinct nucleolus, often with large vacuoles. In the larger cells karyokinetic figures occur in variable numbers; these karyokinetic figures are found sometimes in the centre of young cancerous nests, and sometimes at the periphery. Single direct division of the large cells is the rule. The horny masses in the alveoli and the older parts of the tumour which are already arranged in layers around a centre, have no mitoses.

"The smaller form is found especially in the neighbourhood of large cancer alveoli. The infiltration of small cells described up to the present in the neighbourhood of such cancerous alveoli rests, in our opinion, wholly, or to a large extent, at any rate, on the migration of such young zoospores; the smaller form grows into the larger, which is able to divide. . . ." And further on he states, "A distinction between the parasite cell and the epithelial cell is not possible even at the present time."

Alexis Korotneff,[3] under the name of *Rhopalocephalus carcino-*

[1] Metchnikoff, "Remarks on Carcinomata and Coccidia," *Brit. Med. Journ.* 10th Dec. 1892, p. 1273.

[2] L. Pfeiffer, "Untersuchungen über den Krebs, Die Zell-Erkrankungen und die Geschwulstbildungen durch Sporozoen," Jena, 1893, p. 98.

[3] Alexis Korotneff, "Rhopalocephalus carcinomatosus n. g. und sp. Kor. (Krebsparasite)," *Centralbl. f. Bakteriologie u. Parasitenkunde*, March 1893, Bd. xiii. p. 373.

Korotneff in his paper makes the following remark:—"Oft sind besondere Bildungen zwischen den Carcinomzellen zu finden, die eine Agglomeration von Alveolen mit stark lichtbrechenden Konturen (Wänden) vorstellen; das innere der Alveolen ist schleimig und

matosus, represents structures which he regards as parasitic, and which, according to him, develop from an amœba. The description given by this author applies, as far as we can gather, to carcinoma of the lip only, and is, in many respects, so remarkable that we must defer its discussion to a later paper, which will treat more particularly of these cancers. We would state at once that, up to the present, we have not been able to confirm many of Korotneff's statements, which appear to us to be for the most part erroneous.

Lastly, Jackson Clarke has described in several communications before the Pathological Society of London various appearances illustrating, in his opinion, the formation of spores in the parasites of cancer. We have had occasion to express our opinion on this point before this learned Society, and we can only add that our criticisms on the observer's work have been practically endorsed by the Morbid Growths Committee of the Society. We must state again, however, that neither Dr. Sims Woodhead (in whose laboratory our researches have been carried out, and who has seen a large number of our preparations) nor ourselves have seen in Mr. Clarke's preparations more than a single structure resembling even remotely those described by Walker and ourselves.

In a further communication, Mr. Clarke showed some structures[1] which he had found in a tumour of the cat's lip, and which he designated as psorospermial growths. Professor Boyce and one of us (R.) contended at the meeting that the bodies shown had nothing in common with psorosperms, and Professor Boyce stated that they were probably eggs of nematodes, resembling those found in flukes.

The parasite of cancer, according to our observations, either divides into two, or into multiples of two, the simple division into two parts being the more frequent form of multiplication.

In such cases the nucleus of the parasite first elongates a little so as to become somewhat oval in shape. The nucleus then divides into two absolutely equal parts, a fissure making its appearance exactly in the centre, and gradually deepening (Plate II., Fig. 27, and following). The two nuclei thus formed then gradually separate, though they remain connected for a long time by very fine, delicate, and somewhat granular threads.

The capsule of the parasite shows no changes at first, and the time

färbt sich ganz schwach (fig. 15), es sind leere Cysten von Sporozooiden, die von dem Plasma inhalte verlassen sind und gewöhnlich von lymphatischen Zellen eingenommen werden; ich finde daher die Meinung, die neulich in der Litteratur ausgesprochen ist (Ruffer and Walker) dass es gestorbene Parasiten sind, unhaltbar."

I must remark that this criticism is evidently a misunderstanding on Professor Korotneff's part, as the dead parasites we described were not present between the "carcinomzellen" but were contained in the cells themselves. In the first plate of our paper, Metchnikoff has depicted such a parasite in the interior of a cancer-cell (M. A. R.).

[1] Pathological Society, reported in *Lancet* and *Brit. Med. Journ.* Saturday, 6th May 1893.

at which it undergoes division appears to vary somewhat even in the same cancer. As a rule, however, it begins to divide when the two nuclei are separated from each other, but are still connected by the threads above mentioned (Plate II., Fig. 28). A septum is gradually formed by a prolongation thrown out from either side of the capsule until the two prolongations meet, but even at that time, when the septum is formed, the threads connecting the two nuclei are still plainly seen. It may interest those engaged in microphotographic researches to know that this last stage was first revealed to us by a photograph.

In a later stage the threads disappear; the two parasites lie with their inner parts flattened against one another, then gradually become rounded off until, finally, they separate as two young parasites.

The stage in development at which this division by fission takes place varies greatly, it may be in very small as well as in extremely large parasites. In the large majority of cases, however, it occurs in those of medium size, as illustrated in Plate II., Fig. 27, and the following sketches. In other cancers, more especially abdominal carcinomata, we have observed divisions in some very large parasites.

It is not rare to see in some of the cells several parasites undergoing division at one and the same time, and in this way the cell may include a large number of small parasites.

This form of division of the parasite is the most common, and may be observed in every cancer of the breast; but sometimes, instead of dividing into two, it subdivides into four, six, eight, sixteen, or as many as thirty-two young parasites.

In this form parts of the nucleus become fragmented off, and arrange themselves at the periphery of the parasite, whilst at the same time, or shortly afterwards, a process of segmentation takes place in the capsule. The fragments of the nucleus thus separated again subdivide into several parts, the division of the capsule generally following suit. In this way a body is produced, resembling, to a great extent, the form *en rosace* of the parasite of malaria, as described by Laveran and others. Our friend, Professor Metchnikoff, who examined our preparations, had the kindness to paint Plate III., Fig. 39, for us, which partly illustrates our statement. The cell which he has depicted here contained one more parasite in the act of dividing, which, however, was obscured by the nucleus lying above it, and which, for this reason, was not included in the painting. We beg to thank him for giving us his assistance as one of the most competent of zoologists, and for allowing us to make use of this painting.

Not unfrequently a small part of the nucleus lying in the centre remains behind, and seems to take no part in the division. It resembles, and is possibly identical with, the *corps de reliquat* described in sporozoa.

It is often difficult to make out whether the fragmentation of the nucleus precedes or follows the segmentation of the capsule, but, on the whole, it is probable that the former process is the rule. This does

not take place at one stroke, but often in several stages, according to the size of the parasite. After the periphery has already divided, the centre again subdivides, until the whole of the parasite (except perhaps the *corps de reliquat*) has been used up for the formation of the new parasites. One may often see this appearance in the clusters of young parasites which are formed in this way (Plate III., Figs. 38, 39, 40, 41, 42, 43, 45, 47, and 49).

This mode of division does not result in a number of parasites all contained in the same capsule, *i.e.* a sporocyst, but each young parasite is surrounded by its own independent capsule, and from henceforth leads its own independent life. Each one increases in size and separates from the others, or it may leave the cell to infect another one.

We have diligently sought in each one of the fifty cancers of the breast examined by us for anything resembling crescentic spores, such as are characteristic of sporozoa. We can only say that, although we have observed structures resembling those described by Strœbe, Soudakewitch,[1] Podwyssoszki, and Sawtschenko, we have never been able to trace their formation from the structures we describe as parasites. We therefore fully endorse Metchnikoff's opinion, previously quoted.

On two occasions, however, we have seen, in preparations fixed in Flemming's solution and stained with methyl green and acid fuchsine, crescentic bodies, such as Soudakewitch has depicted in his second paper. So like were these figures that, *mutatis mutandis*, Soudakewitch's[2] drawings might have been taken from one of our preparations. But although these bodies were for the most part crescentic in shape, containing a darker nuclear (?) centre, surrounded by a protoplasm, yet their irregular size and arrangement, and the want of definiteness in the contour of their protoplasm, led us to believe that we were most likely dealing with a product of degeneration, and not, as we had hoped, with a mode of reproduction of the parasite of cancer.

Section IV.[3]

It is our intention in this section to discuss briefly various structures

[1] See Soudakewitch, *loc. cit.* plate xix. fig. 5.

[2] See Soudakewitch's second paper, plate iii. fig. 5.

[3] In "A Preliminary Note on some Parasitic Protozoa found in Cancerous Tumours," published in the *Brit. Med. Journ.* July 16, by Dr. Ruffer and myself, three of the plates illustrating it (figs. 4, 6, and 7) were wrongly described as containing parasites instead of pseudo-parasites. The error is wholly mine. Dr. Ruffer being abroad did not see the proofs, and therefore my hurried and somewhat careless description of these figures was allowed to go to press uncorrected. My whole time latterly having been given to entirely different work, I have been unable to follow closely the discussions on the subject of protozoa in cancers. I find that it is rapidly becoming one of the "burning questions of the day," and, in consequence, wish to clear away any misconstructions, which might easily arise from the fact that we described the same figures as one thing in our preliminary notes, and as another thing in our more detailed paper. My correction coming so long after the error, I wish to make it the more complete. I must repeat, therefore, that Dr. Ruffer was

present in carcinomata of the breast, which have been described as parasites—erroneously in our opinion—by various observers, or which we think might be mistaken for parasites. The difficulties of such criticism are obvious, as, not having seen the preparations of other observers, we must trust ourselves only to descriptions and illustrations, although, on the other hand, some of the papers already enumerated by us have been beautifully illustrated. At the same time, we shall seize the opportunity of answering the criticisms which have been launched against Ruffer and Walker by various observers.

It has been known for some time, especially since the researches of Arnold, that the nuclei in various normal and pathological structures of men and animals undergo a process which Arnold has described under the name of segmentation and fragmentation.[1] Indeed, he states that when this fragmentation of the nucleus takes place, small cells may arise through endogenous formation, around the part of the nucleus which is fragmented off from the rest of the nucleus. Vitalis Müller,[2] a pupil of Arnold, has tried to prove that the bodies described by Ruffer and Walker were nothing more than forms of endogenous cells derived in that way. We will now discuss Müller's contention in detail, as far, at any rate, as it applies to cancer of the breast.

In the first place, Müller assumes as proved the formation of endogenous cells in cancer described by Arnold. He might have added, however, that this formation of endogenous cells is accepted by very few competent microscopists, and that Arnold's observations have been contradicted by more than one competent observer. Denys,[3] for instance, who has repeated Arnold's work, denies that this process occurs, at any rate, as far as the bone-marrow is concerned. Cornil,[4] working independently, is intensely sceptical. Demarquaix[5] is of opinion that the appearances described by Arnold are simply post-

wholly unconscious of the mistake until it appeared in print; but as our full paper was to appear so shortly in the *Journal of Pathology and Bacteriology*, we unfortunately omitted to point out the error. At the time of the publication of this preliminary note the original drawings and paintings of these same figures with their true descriptions were in the hands of Dr. Sims Woodhead [That is the case—Ed.], the Editor of the *Journal of Pathology and Bacteriology*. He had frequently seen all our preparations, and had discussed with us the scope of our paper, and the question of what illustrations should accompany it. J. Herbert Walker, M.A. (Oxon.)

[1] Strœbe ("Kerntheilung und Riesenzellenbildung in Geschwülsten und in Knochenmark," *Beiträge zur allgemeinen Pathologie und pathologischen Anatomie*, 1890, vol. vii. p. 343) has given an excellent account of the state of this question.

[2] Vitalis Müller, "Ueber celluläre Vorgänge in Geschwülsten," *Virchow's Archiv*, 1892, Bd. iv. p. 512.

[3] Denys, "La cystodiérèse des cellules géantes et des petites cellules incolorées de la moelle des os," *La Cellule*, tome ii.

[4] Cornil, "Sur la multiplication des cellules de la moelle des os par division indirecte dans l'inflammation," *Arch. de physiol. norm. et path.* 1887.

[5] Demarquaix, "Quelques remarques à propos du dernier travail d'Arnold sur la fragmentation indirecte," *La Cellule*, 1889, tome v.

mortem changes, whilst Kölliker[1] is not prepared to accept Arnold's explanations.

Lukjanow,[2] discussing the question in his work on the pathology of the cell, asks himself the question: "Kann man in pathologischen Fällen *irgend welche Daten*[3] zu Gunsten der Hypothese von der endogenen Vermehrung finden?"—surely a sceptical frame of mind.

Strœbe,[4] working under Nauwerk's direction, has repeated Arnold's work on the bone-marrow, and has come to the conclusion that the figures described by Arnold under the name of indirect fragmentation of the nucleus are perfectly accurate. With regard to the division of the protoplasm in the giant cells of bone-marrow, however, he is far more cautious, saying: "Auch Anzeichen von Protoplasmatheilung *habe ich* an den Riesenzellen *zu sehen geglaubt*, besonders Einfurschungen vom Rande her." With regard to cancer cells (carcinoma and sarcoma) he has found figures belonging to the scheme of direct and indirect fragmentation of the nucleus as described by Arnold. But with regard to the formation of endogenous cells in other cancer cells, Strœbe is again extremely cautious. He says: "Wie Arnold, könnte ich in einer allerdings beschränkten Anzahl von Fallen Bilder sehen, welche darauf *hinzudeuten schienen*, dass derartige isolirte, wandständig liegende Kerne sich mitsammt dem anliegenden Protoplasma vom alten Zell-leib abfurchten und lostrennten. *Eine Bildung von Tochterzellen im Innern der Mutterzelle habe ich nicht geschen.*"

Such being the case, it was only to be expected that before attacking other observers' work, Müller would first place Arnold's contentions on a more satisfactory basis, but we have in vain looked in his paper for a single fact complementing Arnold's observations. Unable to describe any new facts, Müller tries to bolster up Arnold's contentions by the work of other observers without waiting to consult their latest publication.

Since the beginning of our researches, we have directed our special attention to this question, and we have seen most of the appearances of the nuclei described by Arnold in his numerous papers. For this purpose we have examined bone-marrow and tumours fixed whilst still alive in corrosive sublimate, osmic acid, Foà's solution, Flemming's solution, and absolute alcohol, and stained with the most varied nuclear and protoplasmic colouring reagents—hematoxylin, carmine, saffranin, nuclear black, methyl blue, methyl green, rose bengale, acid fuchsine, eosin, cochineal, etc. Neither in the bone-marrow nor in the malignant tumours did we find any appearances which gave any support to the theory of the endogenous formation of cells, nor indeed to the idea of the direct division of cells. Moreover, Arnold in his descriptions has not excluded the possibility of these so-called endogenous forms being invaginated cells or leucocytes which have been absorbed by the giant cells

[1] Kölliker, "Handbuch," quoted by Strœbe. [2] Lukjanow, "Die Pathologie der Zelle."
[3] The italics in this and other quotations are ours. [4] *Loc. cit.*

of bone-marrow or have penetrated into cancer cells, although their presence in both kinds of structures has been recognised by many observers, as well as by ourselves. However, all the appearances described by Arnold can be accounted for in a different and far more probable manner.

Sheridan Delépine[1] has fallen into the same mistake as Arnold, and has described, as a product of endogenous formation, cells which are clearly invaginated, and which resemble, as he rightly observes, the bodies described by Darier, Albarran, Wickham, Hutchinson, and Bowlby, none of which, by the way, do we consider bear any relation to the parasites described by us. Any doubt as to Professor Delépine's mistake in this matter may be dispelled by reference to p. 681, where he repeats and emphasises his statement.

We must discuss here the appearances presented by invaginated cells, especially as they have by some observers been mistaken for parasites, whilst others, Delépine for instance, have described them as endogenously formed cells. The fact that certain cells appear to be contained in others was first discovered in 1853 by Virchow, who included in the same category a great many heterogeneous structures; but as early as 1868 an assistant of Volkman, Steudener, showed that this appearance was caused by part of some cellular elements being forced into others, so as to be partly surrounded by the latter. As Steudener has proved, they are not really contained in other cells, for by teasing or by examining fresh in salt solution or by other means, the two are easily separated, and the supposed daughter cell is seen to lie in part only in the mother cell.

We have, in Plates III. and IV., Figs. 44, 46, 48, 50, 51, 52, 53, and 59, represented some invaginated cells, after fixing them by various methods and staining with different staining reagents. But practically they all exhibit the same character. They consist of a dark nucleus, stained with the greatest ease with all nuclear dyes, surrounded by a varying quantity of protoplasm. Even when the enclosed cell degenerates (Plate III., Figs. 48 and 50), the remains of chromatin and the coarse protoplasm are sufficiently characteristic to enable us to make the diagnosis. A difficulty arises in the fact that the enclosing cell, through pressure or otherwise, forms a dark border simulating a capsule around the enclosed cell, so that the whole may resemble a parasite lying inside the cyst (Plate IV., Fig. 52; Plate III., Fig. 46, etc.). Moreover, it not unfrequently happens that one of the invaginated cells is pressed into a cell, the nucleus of which is undergoing, or has undergone, the so-called direct fragmentation of Arnold. What evidence have we that cells, such as are figured in Plate IV., Figs. 59 *a* and 60 *a*, are not due to simple invagination, and not to a hypothetical endogenous formation, for which there is not the shadow of a proof?

That such cells have been mistaken for parasites is undoubtedly

[1] Sheridan Delépine, "Protozoa and Carcinoma" (fig. 1), *Brit. Med. Journ.* 1892, vol. ii. p. 674.

true, and we cannot do better than analyse in this respect the paper by Wickham, which has appeared in the *Arch. de méd. exp. d'anat. et path.* 1890. In doing so, our criticisms apply not only to Wickham's work, but also to that of Darier, in whose laboratory Wickham worked, and whose paper he endorsed, and to that of Malassez, who shortly afterwards claimed for himself the priority of the supposed discovery of the supposed parasite of *Molluscum contagiosum*, and explained how he, Malassez, first demonstrated the so-called coccidia of *Molluscum contagiosum* to Darier.[1] If we insist on this fact, it is because in this paper Malassez, speaking of Darier's work on Paget's disease, says: "J'ai bien examiné ses préparations, mais je n'ai eu qu'à confirmer ce qu'il y avait vu;" and so apparently endorses Darier's views; whereas three years later, in a criticism of our work,[2] the same observer expresses himself as follows: "Il est vrai de dire que je suis toujours resté sur une grande réserve touchant la nature de ces formes cellulaires, ne m'avançant réellement qu'au sujet de celles qui ressemblaient le plus à quelqu'une des formes parasitaires bien connues et dont, par suite, la nature était plus évidente et plus certaine; telles sont celles que j'ai trouvées dans l'epithéliome du maxillaire de M. Albarran et dans la psorospermose folliculaire de M. Darier, formes que ces distingués observateurs ont parfaitement décrites depuis," and does not mention Darier's work on the breast. We are all the more unable to understand Malassez's position in this matter as, in the sentence previous to the one first quoted, he attributes to Darier the discovery of the supposed coccidia of Paget's disease. We would notice in passing that up to the present moment we have been unable to satisfy ourselves as to the presence of parasites in *Molluscum contagiosum*.

Now, with regard to the body depicted by Wickham as a typical coccidium (see Wickham, Plate II., Fig. 4), it might have been copied from Plate III., Fig. 51 of our paper. It contains a hard, darkly staining nucleus, with a certain amount of protoplasm around. The capsule corresponds exactly to the pseudo-capsule we have described around these pseudo-parasitic cells. Similarly, Plate III., Fig. 13 D.B., might have been parasitic, copied in Fig. 62 of our Plate. In Plate II., Fig. 5, Wickham has drawn a parasite (?) in which the protoplasm "incompletely retracted, is still adherent to the cyst wall by means of filaments." One might feel inclined to think that possibly these elements might correspond to the radiations described by us, were they not almost identical with the figures described by Steinhaus[3] and others—and in our opinion correctly—as "Carcinomzellen invaginationen."

That the bodies described by us can be sharply distinguished from the

[1] Malassez, "Sur les nouvelles psorospermoses chez l'homme" (*Note Rectificative*), *Arch. de méd. exp. et d'anat. path.* 1890, tome ii. p. 301.

[2] Malassez, "Sur les parasites du cancer," *Compt. rend. de la Soc. de Biologie*, 1893, p. 443.

[3] Steinhaus, "Weitere Beobachtungen über Carcinomeinschlüsse," *Virchow's Archiv*, Bd. cxxvii. p. 173.

pseudo-parasite of Wickham is shown in Plate IV., Fig. 53, in which one of Wickham's pseudo-parasites (*a*) contains one of the bodies which we regard as parasitic (*b*). We would also remark that Borrel,[1] who long ago demonstrated the numerous fallacies contained in Wickham's paper, has found in epitheliomata, bodies closely resembling some of those described by us, of which he speaks as follows :—"Il y a là des formations spéciales qu'il est impossible de rattacher à l'évolution cellulaire. Il ne peut être question ici de formations cellulaires endogènes, de dégénérations de leucocytes introduits dans la cellule," etc. As we are of opinion that the typical coccidium described by Wickham is nothing but an invaginated epithelium cell, we need not discuss further the other appearances described by Wickham as evidence of multiplication, etc. We may add that such bodies may not unfrequently be found in the perfectly healthy skin of man and the ox, and that this appearance can be artificially produced by cutting oblique sections of the epithelium covering the healthy cornea.

Although compelled to express our disagreement with Wickham's explanation of the appearances found in his preparations, we gladly bear testimony to the impulse given by Malassez to the study of parasites in tumours. Indeed, it appears certain that Malassez saw a great deal more than did either Darier or Wickham, as Borrel,[2] who examined his preparations and who overthrew Wickham's work, expresses himself as follows :—"M. Malassez me fit l'honneur de me montrer ses préparations et les figures qui lui avaient suggéré sa remarquable hypothèse. Je fus vivement frappé des figures qu'il me montra ; ce n'étaient pas du tout celles que j'avais crû devoir critiquer." For the same and other reasons we are quite unable to agree with the views expressed by L. Pfeiffer, concerning the appearances which he regards as young parasites.

We may, after this digression, return to Müller's objections, and point out that this author nowhere gives us any inkling as to how we are to distinguish these invaginated cells from the cells which he supposes to have formed endogenously ; and we would remark, by the way, that the figures with which Müller illustrates his paper hardly support his own contentions, and that, quite apart from the parasitic theory, a totally different explanation may be given of all his figures. His technique, like that of Professor Boyce, who trusted to alcohol hardening and hæmatoxylin staining, is extremely deficient. His preparations are overstained, and nowhere have we seen any nuclei of such funereal blackness as are represented in his drawings. They are indeed clad in "the trappings and the suits of woe." Now, although we, like most other observers, have not been able to satisfy ourselves as to the formation of endogenous cells, we would remark that, even allowing that this process occasionally takes place, the

[1] Borrel, "Evolution Cellulaire et parasitisme dans l'épithelioma," p. 24. Montpellier, 1892.

[2] Borrel, *loc. cit.* p. 12.

parasites described by us are totally different from the endogenous cells described by Arnold, or, in fact, from any tissue-cells either formed endogenously or invaginated. The nuclei of the so-called endogenous cells have all the reactions of the nuclei of ordinary cells. They stain darkly with any nuclear dye, after having been hardened in almost anything (Plate IV., Figs. 55, 56, 57, 59, and 60). On the other hand, the nucleus of the parasite is almost "absolut refraktär"[1] to nuclear dyes and even to hæmatoxylin. True, it may take up hæmatoxylin when quite young, as Ruffer and Foà have shown, but even then it frequently shows the phenomenon of metachromatism; and when the section is stained with saffranin and hæmatoxylin, after fixation in Herrmann's fluid, the nucleus of the cell takes up saffranin, the parasite retaining the hæmatoxylin. As soon as the parasite increases in size, it prefers protoplasmic dyes, even when it divides and subdivides. True, by over-staining, etc., it is possible to stain even the parasite with hæmatoxylin, but such over-coloration would spoil all possible differentiation, as may be well illustrated by examining the pictures accompanying Müller's paper. Moreover, in the large majority of cancers of the breast, the parasite is at no time in the nucleus, or even connected with the nucleus, the two being independent from first to last. It would also be interesting to know how Arnold and his pupils would explain the forms of division which we have illustrated in Plate II., Figs. 26, 27, 28, 29, and Plate III., Fig. 39, etc., or such forms as seen in Plate III., Figs. 40, 41, 42, 45, 47, 49, or even such groups as in Plate I., Figs. 5 and 6.

It is quite true, we admit, that one may find in cancer nuclei resembling those described by Arnold, but nowhere have we seen any figures illustrating the supposed formation of daughter cells by endogenous formation.

A fact which in our opinion militates very strongly against Arnold's theory is that, in many cases, the protoplasm of the cells containing such nuclei shows marked degenerative changes instead of changes of a progressive nature, though, on the other hand, we admit that they are often present near actively growing points.

As a matter of fact, therefore, Arnold's theory is not proven, and even if proven, the arguments which his pupil Müller has brought forward in no way invalidate the observations of Ruffer and Walker, simply because the bodies described by Müller are not identical with those depicted by the latter observers.

We now wish to draw attention to certain structures often met with in cancer, and also, we believe, in normal tissues, which are of particular interest because they have, in our opinion, been mistaken for stages in the life history of cancer protozoa. We believe that they resemble greatly some bodies found in physiological tissues, such as those described by Nicolas,[2] who considers them

[1] Burchardt, *loc. cit.*

[2] We were unfortunately unable to obtain Nicolas' original paper.

as a special secretion product of the cells, and by Bizozzero,[1] who regards them as stages of degeneration of leucocytes. Similar and probably identical bodies have been described by Heidenhain[2] in the columnar epithelial lining of the intestinal tract of various animals. Heidenhain also suggests that their origin may be in degenerated leucocytes.

The bodies referred to are small round structures lying in the interior of the protoplasm, and consisting of a chromatic part, which stains well with the ordinary nuclear dyes, surrounded by a layer of protoplasm which takes up the counter-stain (Plate IV., Figs. 64, 66, 67, 68 *a*).

Not unfrequently several small chromatin bodies are scattered through this body, whilst at other times the chromatin has a falciform appearance. They resemble in an extraordinary degree, and are, in our opinion, identical with, some of the bodies described as falciform spores by Soudakewitch and Sawtschenko.[3] They also appear to us to resemble some of the structures described by Podwyssoszki and Sawtschenko, but as these observers adopted staining methods very different from our own, we throw out this hypothesis in a tentative fashion only.

They are often met with near degenerated parts of the tumour, but also not unfrequently in cells undergoing karyokinetic division, so that possibly they may be found to be aberrant fragments of chromatin. They also resemble some of the bodies depicted in so beautiful a manner by Steinhaus[4] in his first paper, and some described by Klebs as degenerated white corpuscles.[5] Their signification is to us somewhat obscure. In spite of many endeavours, we have not been able to trace them to leucocytes, and we cannot therefore accept the theory that they are degenerated leucocytes, though we have little doubt that they have been more than once so mistaken. The small size of their nuclei, the little chromatin globules scattered through them, and other characteristics, differentiate them in many respects from leucocytes undergoing cellular digestion. We have had occasion to compare them with a whole series of preparations from various healthy and pathological tissues (bone-marrow, granulation tissue, intestinal tract, etc.) in which this intracellular digestion of leucocytes is apparent, but at no stage did we see anything absolutely identical with the chromatin bodies just described. Are they, then, some product of the degeneration of epithelial

[1] Bizozzero, "Ueber die schlauchförmigen Drüsen des Magendarm Kanals und die Beziehungen ihres Epithels zu dem Oberflächenepithel der Schleimhaut," *Archiv f. mikr. Anat.* 1892, Bd. lx. Heft 3, pp. 360, 361.

[2] Heidenhain, "Beitrage zur Histologie und Physiologie der Drüsenschleimhaut," *Archiv f. d. ges. Physiol.* 1888, Bd. xliv. Supplementheft, p. 22.

[3] See Soudakewitch, *loc. cit.* plate xii. fig. 18. Sawtschenko, *loc. cit.* Taf. i. fig. 19.

[4] Steinhaus, "Ueber Carcinom-Einschlüsse," *Virchow's Archiv*, Bd. cxxvi. p. 533.

[5] Klebs, "Allgemeine Pathologie," Bd. ii. p. 525.

cells? The fact that they are not unfrequently found near degenerated parts is in favour of this contention; on the other hand, we must remember that they are often seen in perfectly healthy cells, and even in dividing cells. They may possibly be small chromatin bodies thrown off during karyokinesis, which afterwards lead a separate existence. But whatever be their true signification, one thing appears to us certain, namely, that they have nothing to do with the parasites which we have described. They may be found in large numbers in cancers which contain few parasites and, conversely, the cancer examined by us which showed most parasites did not contain a single one of them. When both parasites and these bodies were present in the same cancer they were hardly ever present together in the same cell, and we never saw one of these bodies within the cyst wall surrounding the parasite. Moreover, the presence of a corpuscle staining so easily with any nuclear dye, is quite enough to differentiate them sharply from the parasites described by us.

We must now discuss certain other appearances which may be found in cancer, and which resemble to some extent the parasites which form the primary subject of this paper. We refer more particularly to the appearances described as hyperchromatosis of the nuclei, and to degenerative changes in the nuclei and in the cell-protoplasm.

Under the term of hyperchromatosis, Klebs has described an appearance met with in cancer and sarcoma cells in which the nucleus contains a large number of chromatic granules of different sizes. He is of opinion that this hyperchromatosis is due to the breaking up of leucocytes and the absorption of the nuclear substance of the latter into the chromatin of the cancer cells. We need not here repeat the objections which have been brought against this mode of interpretation of these phenomena;[1] it should be observed, however, that hyperchromatic nuclei are by no means always present in carcinoma, and although they may be demonstrated in tissues hardened in Flemming's solution, they are perhaps best seen in fresh cover-glass preparations appropriately stained.

But the hyperchromatosis is far from being a change of a progressive character, and in this we agree with Strœbe, who thinks that it is rather a sign of degeneration. Nevertheless, the contents of these nuclei, consisting of bodies staining intensely with aniline dyes, might be mistaken for parasites, if their intense coloration and their very irregular size and arrangement were not sufficient to distinguish them from an intranuclear stage of a parasite. We would in this respect draw special attention to the observations of Steinhaus, though we are inclined to believe that some of the bodies described by him were of parasitic origin.

It sometimes happens that a body forms in the lumen of an alveolus surrounded by cancer cells, and this body may become surrounded by a pseudo-membrane. One can easily see that this body is merely a sort

[1] See Strœbe, *loc. cit.* p. 25; also Ruffer and Walker, *loc. cit.*

of cast containing the excretions of the cancer cells. It contains homogeneous granules of varying size, which have been formed in the cells and are thrown off into the centre space of the alveolus (Plate IV., Figs. 54 and 61 *a*). Should the central cells of the alveolus also degenerate, a granular mass is formed which may be perfectly round, very sharply separated from the healthy tissue around, and which will stain very deeply with saffranin for instance. We have little doubt that some of the bodies described as parasites by certain authors have been formed in this way.

We have purposely abstained from entering into the question of the parasites as etiological factors, reserving this part of our subject until we have finished the examination of the cancer material at our disposal, from both man and animals.

DESCRIPTION OF PLATES.

Plate I.

Fig. 1.—Fixed in Foà's solution. Cancer cell, *overstained* with hæmatoxylin, containing 3 cancer parasites. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 2.—Fixed in chromicised spirit, and stained with gentian violet and acid fuchsine. Cancer cell containing 2 parasites. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 3.—Hæmatoxylin and cochineal staining. *a*. parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 4.—Methyl green and Biondi staining. Cancer cell containing a parasite *a*. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 5.—Several parasites contained in one cell. Cochineal staining. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 6.—Fixed in Foà's solution, and acid fuchsine staining. A group of parasites in a single cancer cell. The nucleus of the cell could be seen below but was not painted in. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 7.—Fixed in chromicised spirit, and stained with hæmatoxylin and cochineal. *a*. Two parasites. The nucleus of the cell is not shown in the figure. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 8.—Four parasites from an unstained preparation fixed in chromicised spirit. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 9.—Fixed in chromicised spirit; gentian violet and acid fuchsine staining. At *a*, a parasite breaking into a mass of round granules, without showing any other signs of division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 10.—Fixed in chromicised spirit; hæmatoxylin and cochineal staining. *a*. Parasite which has broken up into a number of coarse homogeneous clumps. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 11.—Fixed in chromicised spirit, stained with hæmatoxylin and cochineal. *a*. Large parasite undergoing fragmentation without any sign of division. *b*. Small parasite. *n*. Nucleus of cancer cell. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 12.—Stained with hæmatoxylin and cochineal. *a*. Small parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

Fig. 13.—Hæmatoxylin and cochineal staining. Cancer cell containing—*a*. Parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 14.—Hardened in chromicised spirit, and stained with hæmatoxylin and cochineal. *a.* Large parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 15.—Fixed in Foà's solution, stained with gentian violet and acid fuchsine. Two parasites, stained with fuchsine. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 16.—Cancer cell containing a parasite. *a.* The nucleus of the parasite has broken up into several fragments, but there are no indications of a division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 17.—Fixing in chromicised spirit. Hæmatoxylin and cochineal staining. *a.* Parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

PLATE II.

FIG. 18.—Fixed in chromicised spirit. Hæmatoxylin and cochineal staining. The parasite shows the peripheral arrangement of protoplasmic granules. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 19.—Fixation, staining and magnification as before. The capsule of the parasite (*a*) has shrunk in the interior of the cell.

FIGS. 20, 21.—Two parasites from an unstained preparation, fixed in chromicised spirit. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 22.—Fixed in chromicised spirit, stained with hæmatoxylin and cochineal. *a.* Parasite with wrinkled capsule. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 23.—Hardened in chromicised spirit, stained with gentian violet and acid fuchsine. Peripheral arrangement of granules. No sign of division. The nucleus of the cell has not been painted in. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 24.—Fixed in Foà's solution, stained with hæmatoxylin and cochineal. *a.* Parasite with symmetrical arrangement of granules at the periphery. No sign of division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 25.—A parasite from an unstained preparation fixed in chromicised spirit.

FIG. 26.—Cancer parasite undergoing division. The two parts of the nucleus are still joined by threads. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 27.—Stained with hæmatoxylin and cochineal. *a.* Parasite undergoing simple division. The nucleus is fully divided, the capsule only just beginning to divide. Zeiss Oc. comp. 4, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 28.—Hæmatoxylin and cochineal staining. Cancer cell containing—*a.* Parasite undergoing division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 29.—Cancer cell containing—*a.* Parasite undergoing division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 30.—Stained with hæmatoxylin and cochineal. *a.* Parasite which has just undergone division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 31.—Last stage of division. Two parasites still partly joined together. Eosin and aniline blue staining. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 32.—Fixed in osmic acid, and stained with hæmatoxylin. Multiple division and subdivision of parasite. At *a* the subdivision is not complete. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 33.—Fixed in Foà's solution, gentian violet and acid fuchsine staining. *a.* Parasite, probably undergoing degeneration. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 34.—Fixed in chromicised spirit. Hæmatoxylin and cochineal staining. Cancer cell containing parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 35.—Fixed in chromicised spirit, eosin and aniline blue staining. *n.* Nucleus of cancer cell. Zeiss, comp. Oc. 4, Obj. Oil Imm. $\frac{1}{12}$.

PLATE III.

FIG. 36.—Compare No. 9. Very high magnification. Zeiss Oc. 12, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 37.—Hæmatoxylin and cochineal staining. Cancer cell containing—*a.* Parasite in first stage of division. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 38.—Fixing in Foà's solution, gentian violet and acid fuchsine staining. Segmentation of parasite. The nucleus of the cell is not shown in the figure. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 39.—From a painting by Dr. Metchnikoff. Cancer cell containing 8 parasites, 5 of which are undergoing division and segmentation. Zeiss comp. Oc. 8, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 40.—Cochineal and hæmatoxylin staining. The nucleus of the cell is not shown in the figure. *a.* Fragmentation of the nucleus of the parasite. At the periphery the capsule is seen to divide also. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 41.—Fixing in chromicised spirit; gentian violet and acid fuchsine staining. Cell containing a parasite undergoing fragmentation. The capsule has not yet begun to divide. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 42.—Fixed in chromicised spirit, and stained with hæmatoxylin and cochineal. Cancer cell containing a segmenting parasite. Zeiss Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 43.—Fixed in chromicised spirit; cochineal staining. A cancer cell containing—*a.* Small parasite; *b.* a large parasite undergoing division and segmentation; *c.* a small intranuclear parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 44.—Fixed in Flemming's solution; methyline green and Biondi staining. *a.* Invaginated cell, or pseudo-parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 45.—Fixed in chromicised spirit, and stained with hæmatoxylin and cochineal. *a.* Division and segmentation of parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 46.—Fixing in chromicised spirit; hæmatoxylin and cochineal staining. Pseudo-parasite identical with parasite of Wickham. *a.* Points to the pseudo-capsule. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 47.—Parasite undergoing division and segmentation of capsule. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 48.—Fixing in chromicised spirit; hæmatoxylin and cochineal staining. *a.* Degenerating invaginated cell or parasite of Wickham. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 49.—One parasite dividing and subdividing into a number of younger parasites. At *a* the division is not yet complete. Hæmatoxylin and cochineal staining. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 50.—Fixed in chromicised spirit, and stained with hæmatoxylin and cochineal. *a.* An invaginated and degenerating cancer cell or parasite of Wickham. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 51.—Fixing in chromicised spirit; hæmatoxylin and cochineal staining. *a.* Invaginated cell, identical with parasite of Wickham. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 52.—Fixed in osmic acid and stained with hæmatoxylin. A compound invagination of cells. *a.* Innermost invaginated cell, simulating a parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

PLATE IV.

FIG. 53.—Methyl green and Biondi staining. Cancer cell *a*, containing an invaginated cell or pseudo-parasite *b*, which contains real parasite *c*. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 54.—Fixed in chromicised spirit, and darkly stained with hæmatoxylin and cochineal. Part of cancer alveolus, containing a sharply defined degenerated area *a*. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 55.—Fixing in Flemming's solution ; methyl green and Biondi staining. Beginning of direct fragmentation of nucleus. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 56.—Hardening in Flemming's solution ; methyl green and Biondi staining. Nucleus undergoing direct fragmentation. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 57.—Hardening in Flemming's solution ; methyl green and Biondi staining. Nucleus undergoing direct fermentation. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 58.—From a preparation by Dr. H. Snow. A hypertrophic, hyperchromatic, and fragmenting nucleus. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 59.—Fixed in osmic acid, and stained with hæmatoxylin. A cancer cell, the nucleus of which has undergone extreme direct fragmentation. *a*. An endogenous (?) cell. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 60.—From a preparation by Dr. H. Snow. A large hypertrophied cell containing a hypertrophic nucleus *n*, and one or more invaginated cells *a*. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 61.—Fixed in chromicised spirit, and stained with hæmatoxylin and cochineal. Part of cancer alveolus : *a*. represents a cast formed by the degeneration of cancer cells ; *b*. a granule on the point of being discharged. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 62.—Stained with methyl green and rose bengale. *a*. An invaginated cancer cell simulating a parasite. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 63.—Fixed in osmic acid, and stained with hæmatoxylin. Marked hypertrophy and degeneration and hydrops of the cell. Direct fragmentation of the nucleus at *a*. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 64.—Stained with methyl green and fuchsine. *a*. Pseudo-parasite. *n*. Nucleus of cell. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 65.—Fixing in chromicised spirit ; eosin and aniline blue staining. *a*. Hyperchromatic nucleus lying in an hypertrophied cell. Compare with *b*, normal cancer cells. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 66.—From a cancer hardened in alcohol, containing at *a*, chromatine bodies. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 67.—Fixed in Flemming's solution, and stained with methyl green and Biondi's reagent. Beginning fragmentation of the nucleus. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

FIG. 68.—Fixed in Flemming's solution and stained with methyl green and Biondi's reagent. At *a* are two chromatine granules, mistaken by some authors for parasites. Vérick Oc. 1, Obj. Oil Imm. $\frac{1}{12}$.

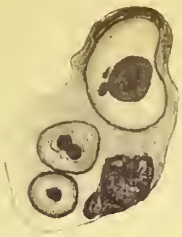
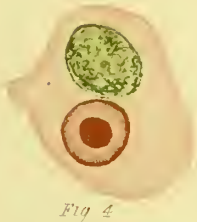


Fig 1

Fig 2

Fig 3



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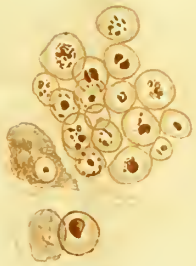

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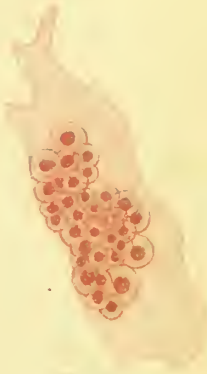

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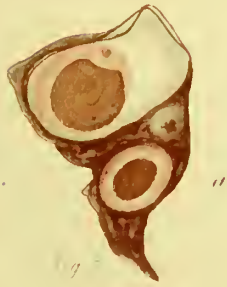

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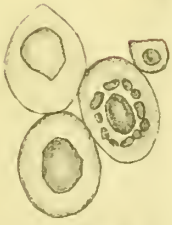

Fig 8

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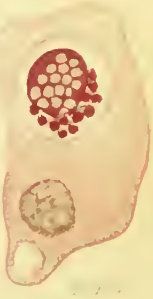

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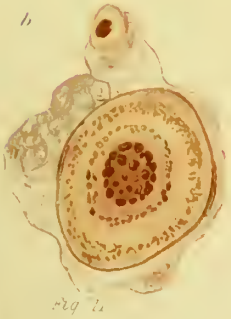
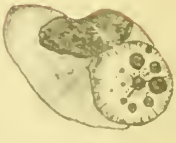


Fig 11

Fig 12

Fig 13



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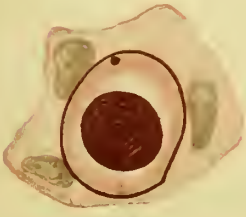

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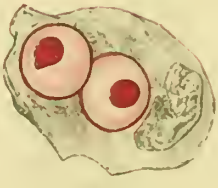


Fig 16

Fig 17

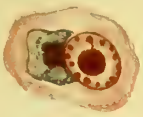


Fig 16

Fig 17

Fig 20

Fig 21

Fig 22

Fig 24

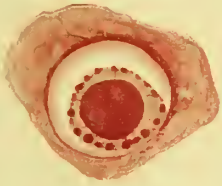


Fig 23

Fig 26

Fig 25

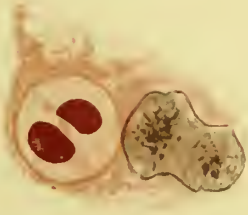


Fig 27

Fig 29

Fig 28

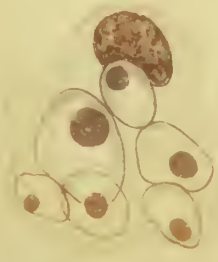


Fig 35

Fig 34

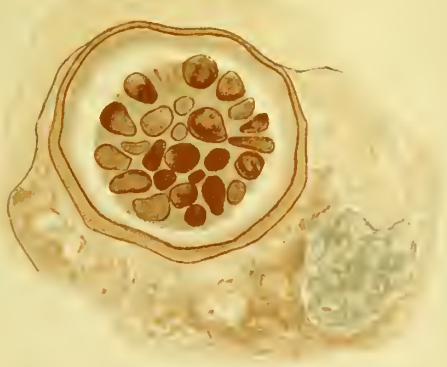
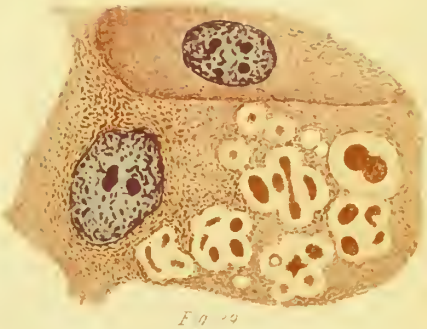


Fig 36

Fig 37

Fig 38



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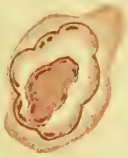


Fig 40

Fig 41

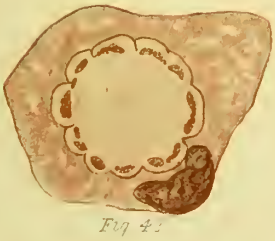


Fig 42

Fig 43

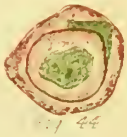


Fig 44

Fig 45

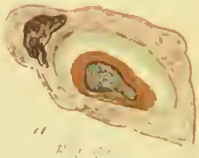
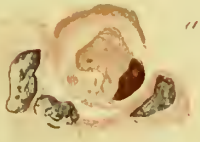


Fig 46

Fig 47



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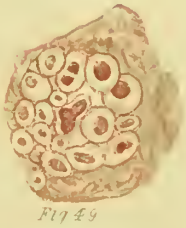

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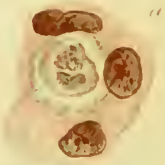

Fig 51